

WHAT IS CLAIMED IS:

- 1                   1.       A method of refolding a first insoluble, recombinant, eukaryotic  
2 glycosyltransferase, wherein the glycosyltransferase comprises a maltose binding protein  
3 domain (MBD), the method comprising the steps of  
4                   (a) solubilizing the insoluble, recombinant, eukaryotic glycosyltransferase in a  
5 solubilization buffer; and  
6                   (b) contacting the soluble eukaryotic glycosyltransferase with a refolding  
7 buffer comprising a redox couple to refold the eukaryotic glycosyltransferase, wherein the  
8 refolded eukaryotic glycosyltransferase catalyzes the transfer of a sugar from a donor  
9 substrate to an acceptor substrate.
- 1                   2.       The method of claim 1, wherein the first eukaryotic glycosyltransferase  
2 is truncated to remove all or a portion of a stem region.
- 1                   3.       The method of claim 1, wherein an unpaired cysteine in the first  
2 eukaryotic glycosyltransferase is removed by substitution with a non-cysteine amino acid.
- 1                   4.       The method of claim 1, wherein the first eukaryotic glycosyltransferase  
2 is selected from the group consisting of GnT1, GalT1, StIII Gal3, St3GalI, St6 GalNAcT1,  
3 Core GalIT1, GalNAcT2.
- 1                   5.       The method of claim 1, wherein the first eukaryotic glycosyltransferase  
2 further comprises a purification domain selected from the group consisting of a starch  
3 binding domain, a thioredoxin domain, a SUMO domain, a poly-His domain, a myc epitope  
4 domain, and a glutathione-S-transferase domain.
- 1                   6.       The method of claim 1, wherein the first eukaryotic glycosyltransferase  
2 further comprises a self cleaving domain.
- 1                   7.       The method of claim 1, wherein the first eukaryotic glycosyltransferase  
2 is expressed in a bacterial host cell as an insoluble inclusion body.
- 1                   8.       The method of claim 1, wherein a second insoluble, recombinant  
2 eukaryotic glycosyltransferase is refolded with the first eukaryotic glycosyltransferase.

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1                   9.       The method of claim 8, wherein a third insoluble, recombinant  
2 eukaryotic glycosyltransferase is refolded with the first eukaryotic glycosyltransferase and  
3 the second eukaryotic glycosyltransferase.

1                   10.      The method of claim 1, wherein the redox couple is selected from the  
2 group consisting of reduced glutathione/oxidized glutathione (GSH/GSSG) and cysteine/  
3 cystamine.

1                   11.      The method of claim 1, wherein the acceptor substrate is selected from  
2 a protein, a peptide, a glycoprotein, and a glycopeptide.

1                   12.      The method of claim 1, wherein the first eukaryotic glycosyltransferase  
2 is a sialyltransferase.

1                   13.      The method of claim 12, wherein the sialyltransferase is selected from  
2 the group consisting of StIII Gal3, St3GalI, St6 GalNAcT1.

1                   14.      The method of claim 12, wherein the donor substrate is a CMP-sialic  
2 acid PEG molecule and the acceptor substrate is selected from a protein, a peptide, a  
3 glycoprotein, and a glycopeptide.

1                   15.      A recombinant, eukaryotic glycosyltransferase, wherein a stem anchor  
2 region and a transmembrane domain are deleted from the recombinant, eukaryotic  
3 glycosyltransferase, and wherein the glycosyltransferase is fused in frame to a maltose  
4 binding domain.

1                   16.      The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2 all or a portion of the stem region is deleted.

1                   17.      The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2 an unpaired cysteine in the recombinant, eukaryotic glycosyltransferase is removed by  
3 substitution with a non-cysteine amino acid.

1                   18.      The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2 the glycosyltransferase is selected from the group consisting of a GnT1 protein, a GalT1  
3 protein, an StIII Gal3 protein, an St3GalI protein, an St6 GalNAcT1 protein, a Core GalIT1  
4 protein, and a GalNAcT2 protein.

1                   19.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2     the glycosyltransferase is a GnT1 protein.

1                   20.     The GnT1 protein of claim 19, wherein the GnT1 protein is a truncated  
2     human GnT1 protein selected from GnT1  $\Delta$ 35 and GnT1 $\Delta$ 103.

1                   21.     The GnT1 protein of claim 19, wherein the GnT1 protein is a human  
2     GnT1 protein comprising an unpaired cysteine substitution selected from the group consisting  
3     of CYS121ALA, CYS121ASP, and ARG120ALA, CYS121HIS.

1                   22.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2     the glycosyltransferase is a GalT1 protein.

1                   23.     The GalT1 protein of claim 22, wherein the GalT1 protein is a  
2     truncated bovine GalT1 protein selected from GalT1  $\Delta$ 70 and GalT1  $\Delta$ 129.

1                   24.     The GalT1 protein of claim 22, wherein the GalT1 protein is a bovine  
2     GalT1 protein comprising an unpaired cysteine substitution of CYS342THR.

1                   25.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2     the glycosyltransferase is an ST3GalIII protein.

1                   26.     The ST3GalIII protein of claim 25, wherein the ST3GalIII protein is a  
2     truncated rat ST3GalIII protein selected from ST3GalIII  $\Delta$ 28, ST3GalIII  $\Delta$ 73, ST3GalIII  $\Delta$ 85  
3     and ST3GalIII  $\Delta$ 86.

1                   27.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2     the glycosyltransferase is a Core1 GalT1 protein.

1                   28.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2     the glycosyltransferase is an ST3Gal1 protein.

1                   29.     The ST3Gal1 protein of claim 28, wherein the ST3Gal1 protein is a  
2     truncated human ST3Gal1 protein selected from ST3Gal1  $\Delta$ 29, ST3Gal1  $\Delta$ 45, and ST3Gal1  
3      $\Delta$ 56.

1                   30.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2     the glycosyltransferase is an ST6GalNAc1 protein.

1                   31.     The recombinant, eukaryotic glycosyltransferase of claim 15, wherein  
2 the glycosyltransferase is an GalNAcT2 protein.

1                   32.     The GalNAcT2 protein of claim 31, wherein the GalNAcT2 protein is  
2 a truncated human GalNAcT2 protein selected from GalNAcT2  $\Delta$ 40, GalNAcT2  $\Delta$ 51,  
3 GalNAcT2  $\Delta$ 74 and GalNAcT2  $\Delta$ 95.

1                   33.     A method of remodeling a protein, a peptide, a glycoprotein, or a  
2 glycopeptide using the recombinant, eukaryotic glycosyltransferase of claim 15.

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